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Flow Cytometric Analysis of HLA-A2+ Donor T Cells for HLA-A2 CMVpp65+ T Cells: Day 0
of Culture

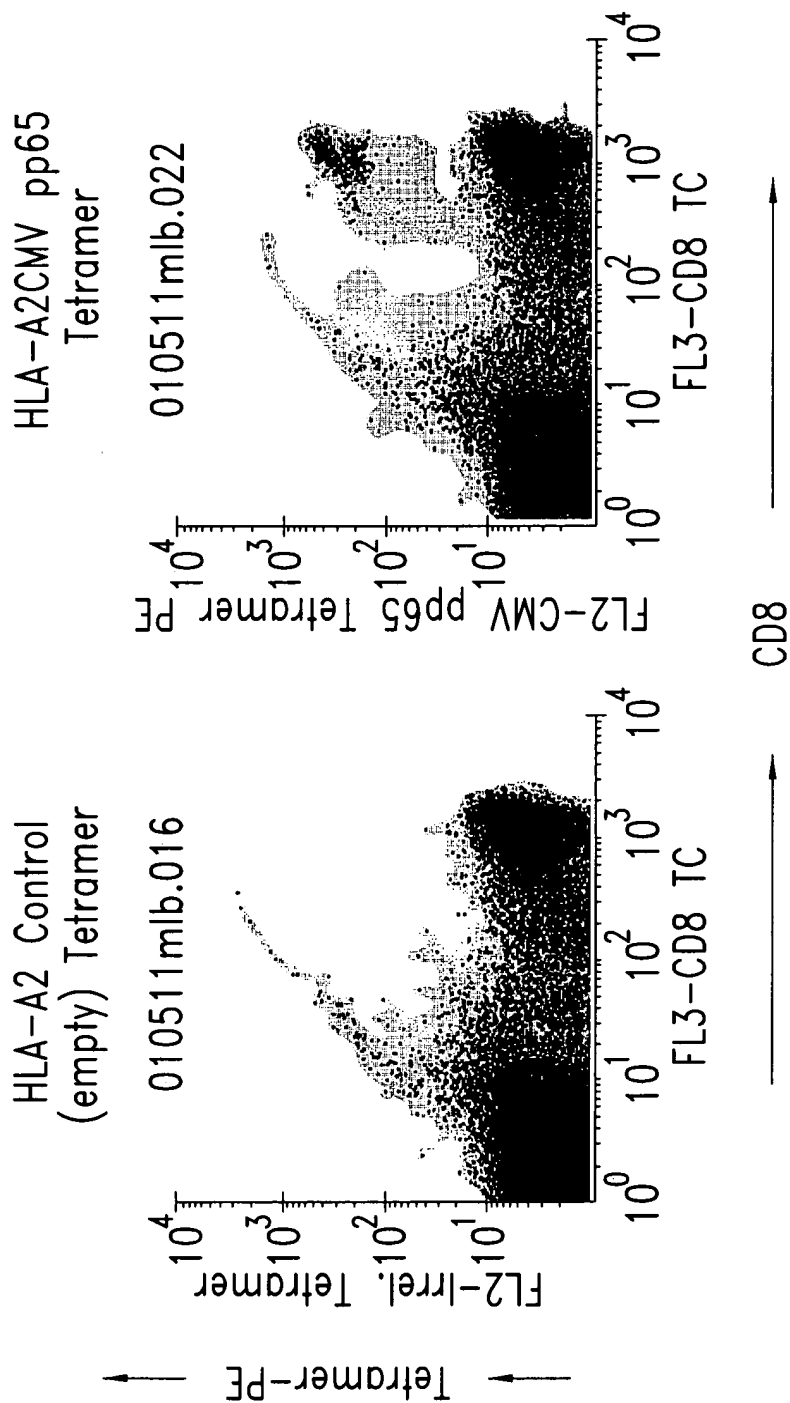


FIG. 1A

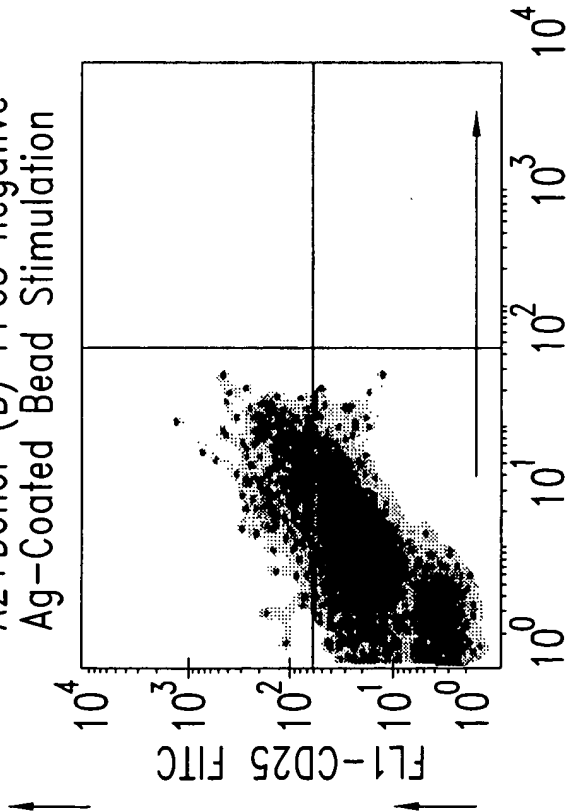
FIG. 1B

Human PBMC were screened for HLA-A2 positivity. HLA-A2+ donors were screened with control (empty) HLA-A2 tetramers and CMVpp65 loaded tetramers. In the donor shown above, approximately 3% of the CD3+CD8+ express TCR specific for HLA-A2 CMVpp65.

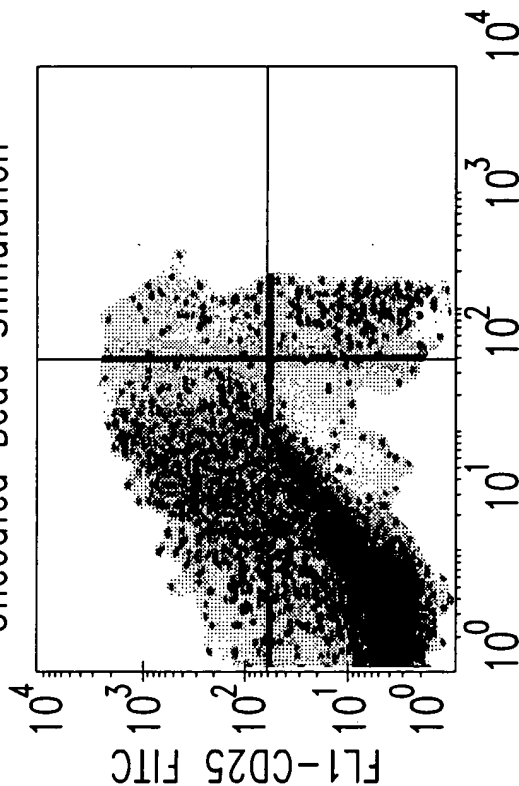


Flow Cytometric Analysis of CD25 Expression on HLA-A2 CMVpp65+ T Cells: Day 10 of Culture

Ag-Coated Bead (Donor 1)
A2+Donor (B)=PP65 negative
Ag-Coated Bead Stimulation



Uncoated Bead (Donor 2)
A2+Donor (B)=PP65 positive
Uncoated Bead Stimulation



FL2-CMVPP65 HLA-A2 Tetramer PE

FL2-CMVPP65 HLA-A2 Tetramer PE

HLA-A2 CMV pp65-PE

(Tetramer Staining Gated on CD8+ T Cells)

PBMC were activated with CMV antigen (coated onto paramagnetic beads) and by day 10 of culture, many cell are shown to be CD25 (IL-2R) positive, and all of the HLA-A2 CMVpp65+ T cells are expressing high levels of CD25, indicating activation (FIG. 2C). Controls include the same donor cells treated with uncoated (antigen-negative) beads (FIG. 2B), or an HLA-A2+ donor (donor 1) that did not show detectable tetramer+ cells at day 0 and was serologically negative for CMV (FIG. 2A). These data indicate that tetramer approaches can be effectively used to track antigen-specific T cells and their relative state of activation.

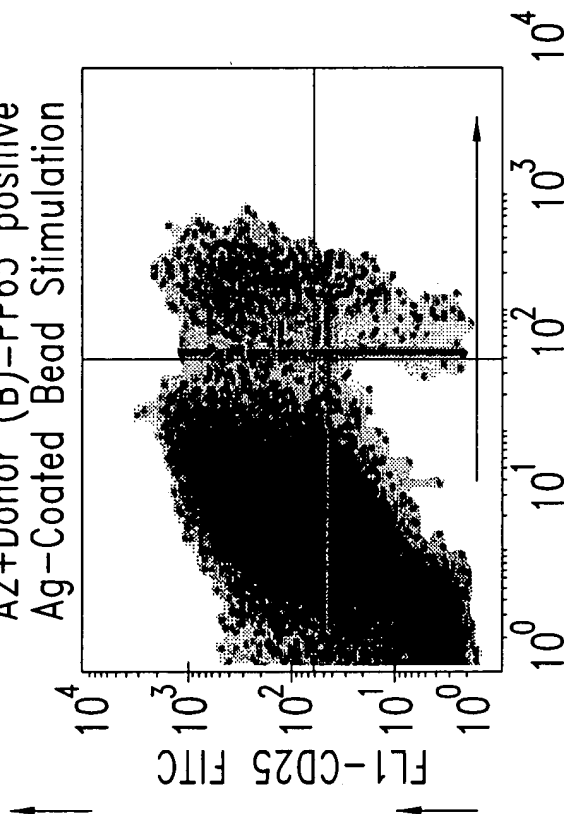
FIG. 2A

FIG. 2B



Flow Cytometric Analysis of CD25 Expression on HLA-A2 CMVpp65+ T Cells: Day 10 of Culture

Ag-Coated Bead (Donor 2)
A2+Donor (B)=PP65 positive
Ag-Coated Bead Stimulation



FL2-CMVPP65 HLA-A2 Tetramer PE

→ HLA-A2 CMV pp65-PE →

(Tetramer Staining Gated on CD8+ T Cells)

PBMC were activated with CMV antigen (coated onto paramagnetic beads) and by day 10 of culture, many cell are shown to be CD25 (IL-2R) positive, and all of the HLA-A2 CMVpp65+ T cells are expressing high levels of CD25, indicating activation (FIG. 2C). Controls include the same donor cells treated with uncoated (antigen-negative) beads (FIG. 2B), or an HLA-A2+ donor (donor 1) that did not show detectable tetramer+ cells at day 0 and was serologically negative for CMV (FIG. 2A). These data indicate that tetramer approaches can be effectively used to track antigen-specific T cells and their relative state of activation.

FIG. 2C

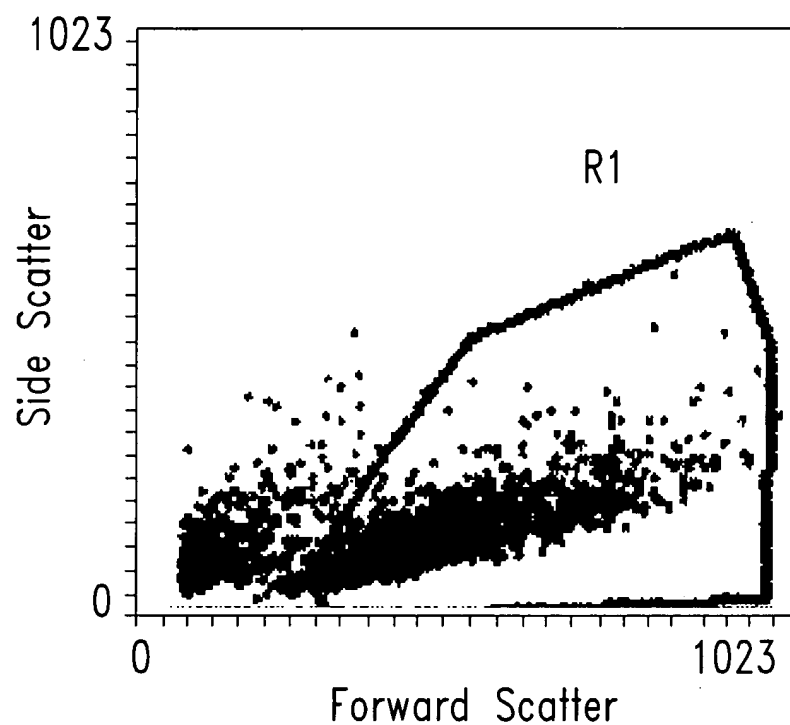


FIG. 3A1

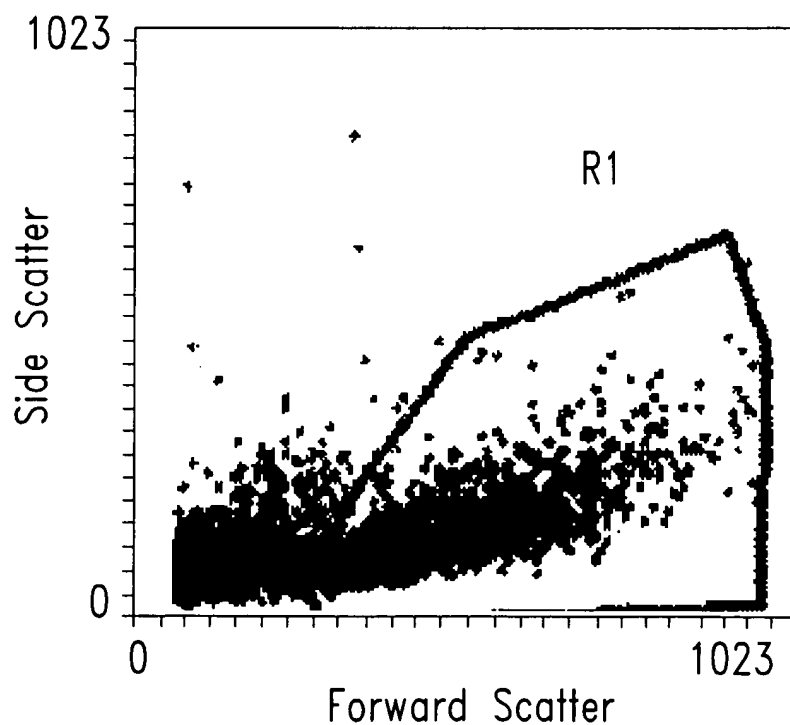


FIG. 3B1

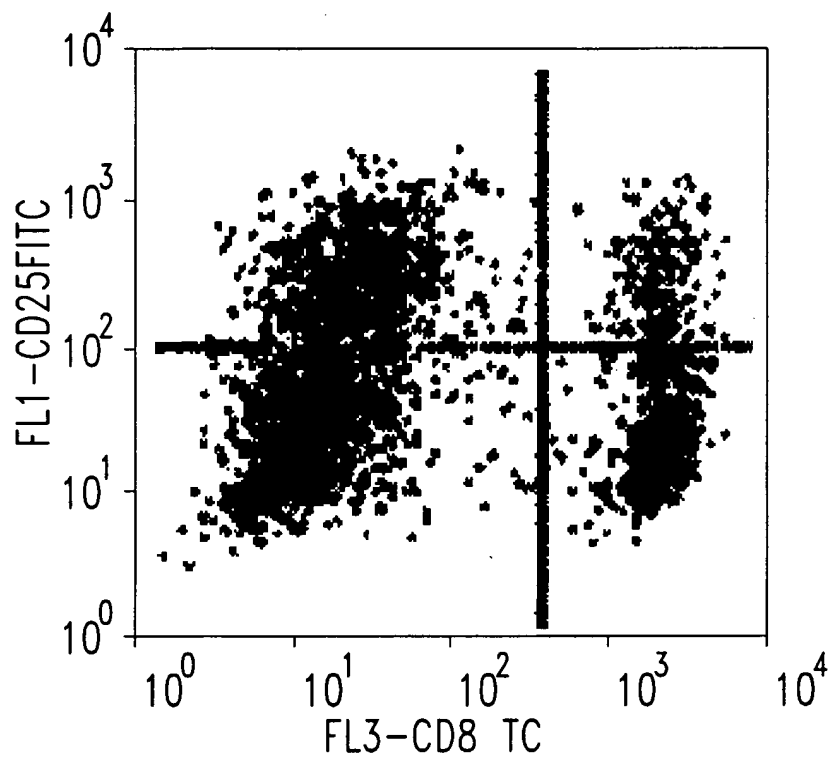


FIG. 3A2

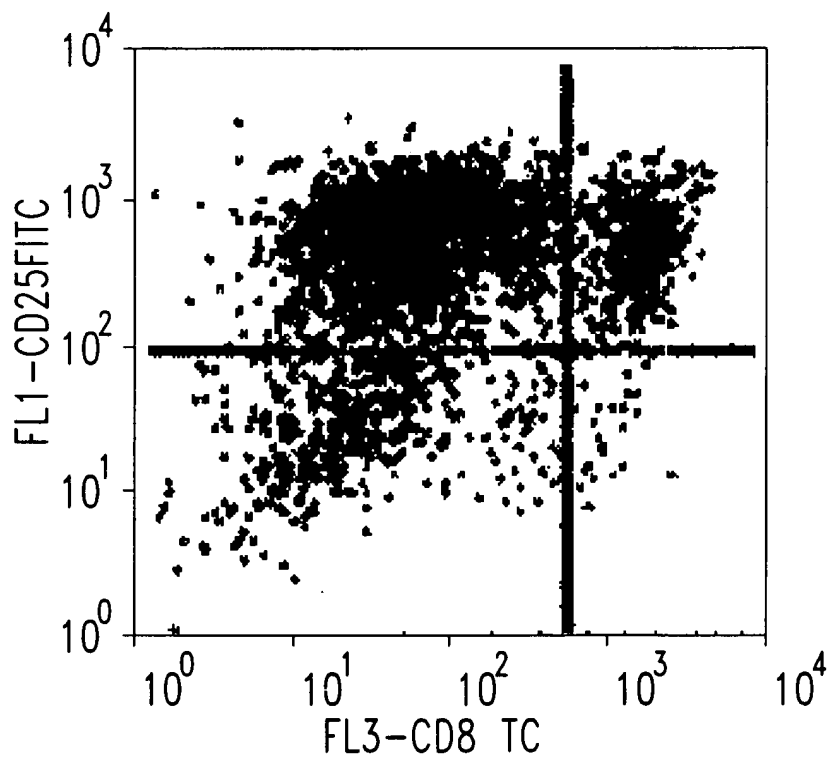


FIG. 3B2

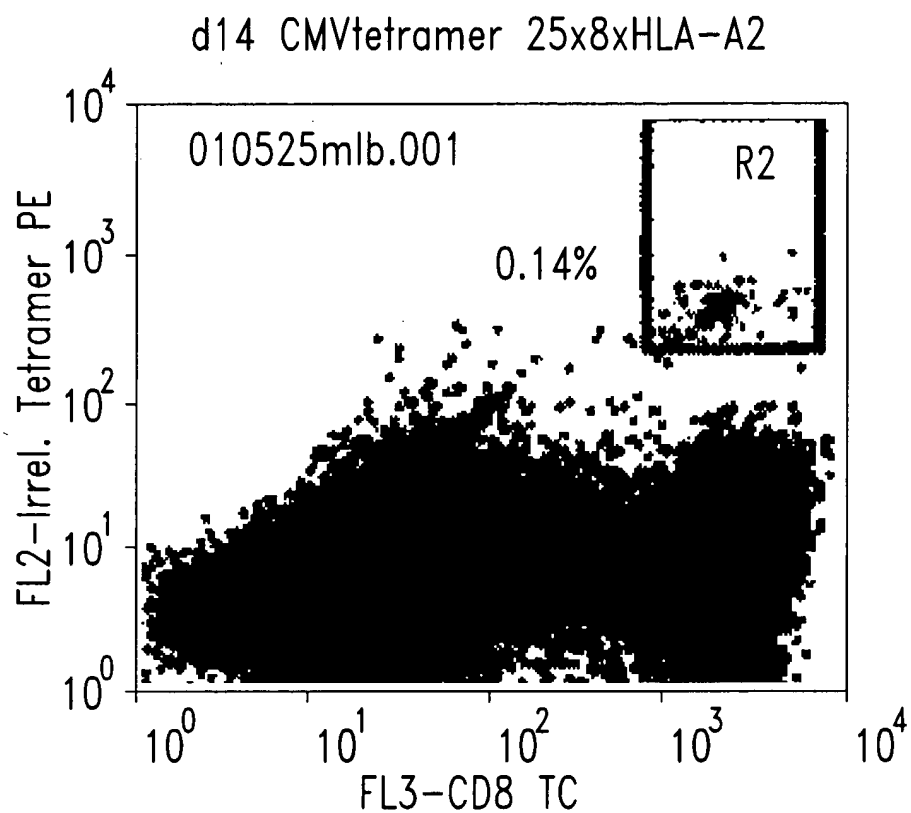


FIG. 3A3

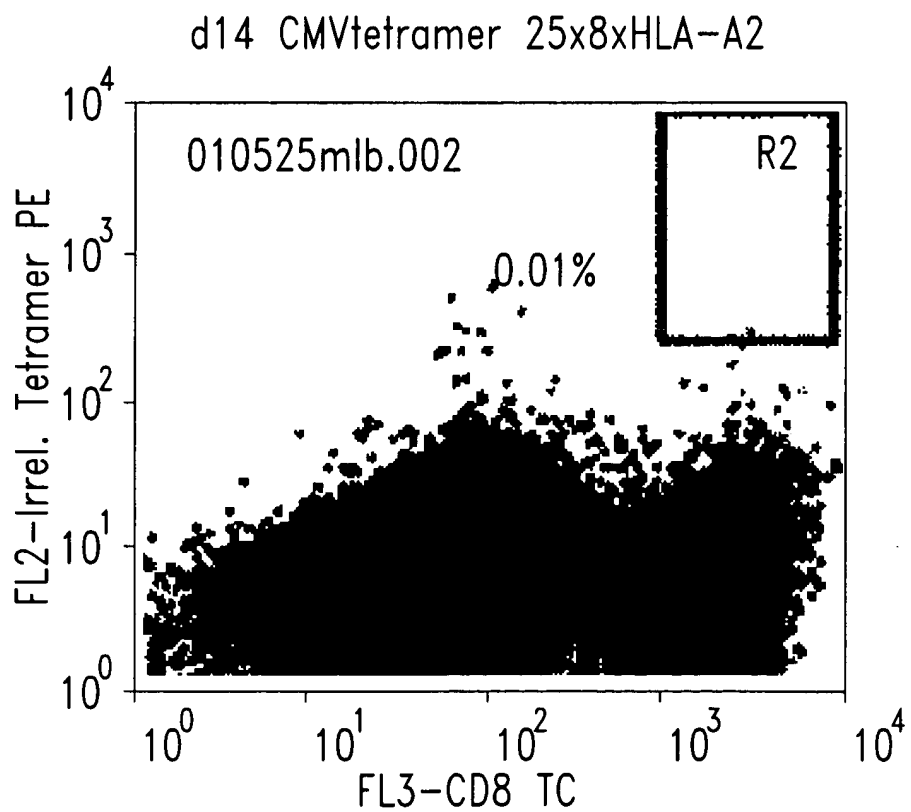


FIG. 3B3

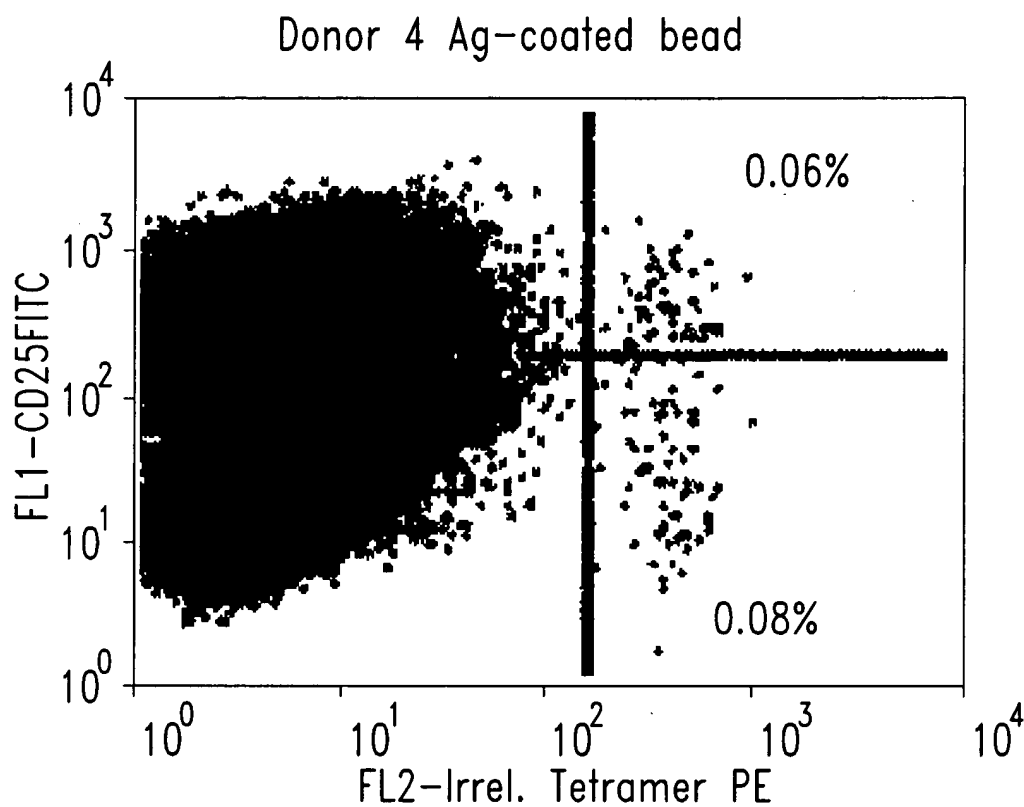


FIG. 3A4

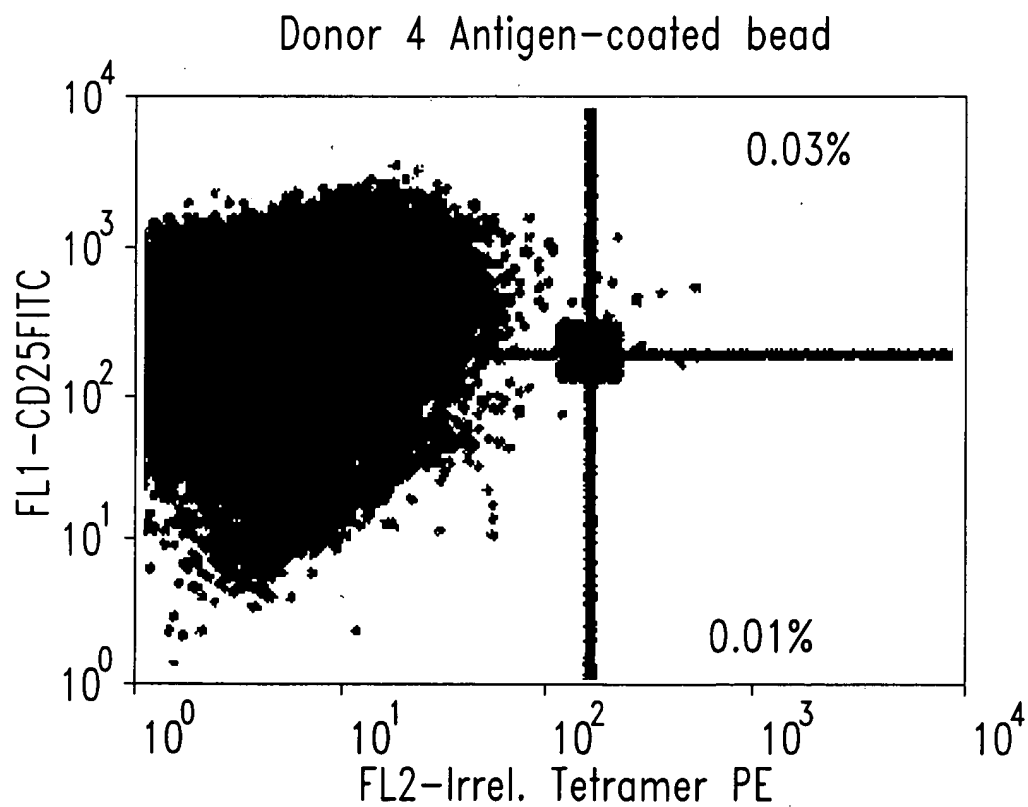


FIG. 3B4



Mixing Xcellerated T Cells with Autologous B-CLL Leukemic Cells Results in the Rapid Upregulation of Key Immunological Effector Molecules Day 12 Xcellerated T Cells Co-cultured 24 hours with autologous leukemic B Cells

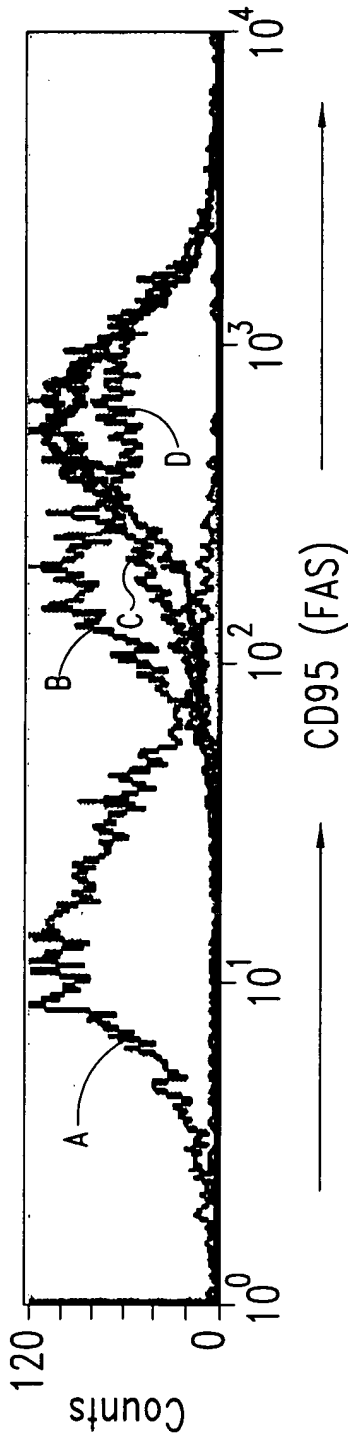


FIG. 4A

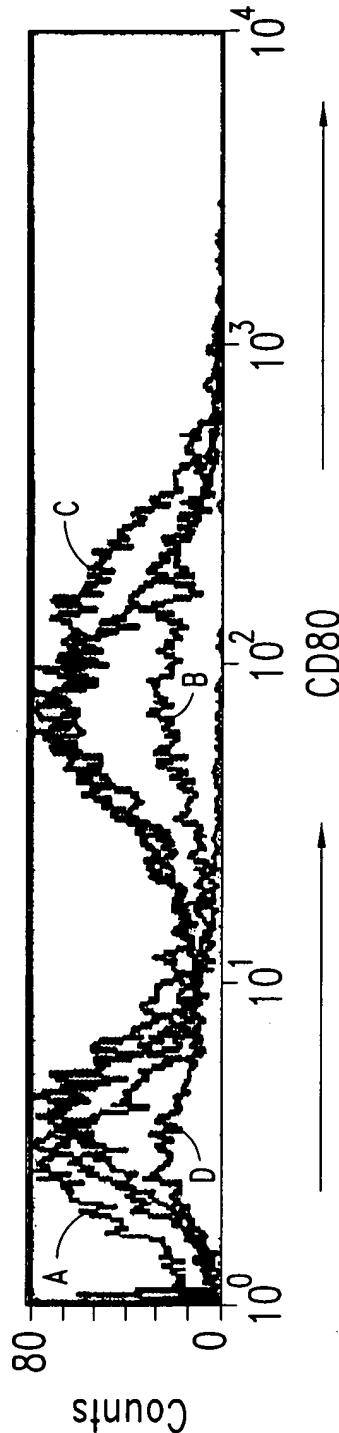


FIG. 4B

A= Leukemic B Cells Alone	B= Leukemic B Cells + Xcellerated T Cells T:B ratio=0.3:1	C= Leukemic B Cells + Xcellerated T Cells T:B ratio=1:1	D= Leukemic B Cells + Xcellerated T Cells T:B ratio=3:1
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Mixing Xcellerated T Cells with Autologous B-CLL Leukemic Cells Results in the Rapid Upregulation of Key Immunological Effector Molecules Day 12 Xcellerated T Cells Co-cultured 24 hours with autologous leukemic B Cells

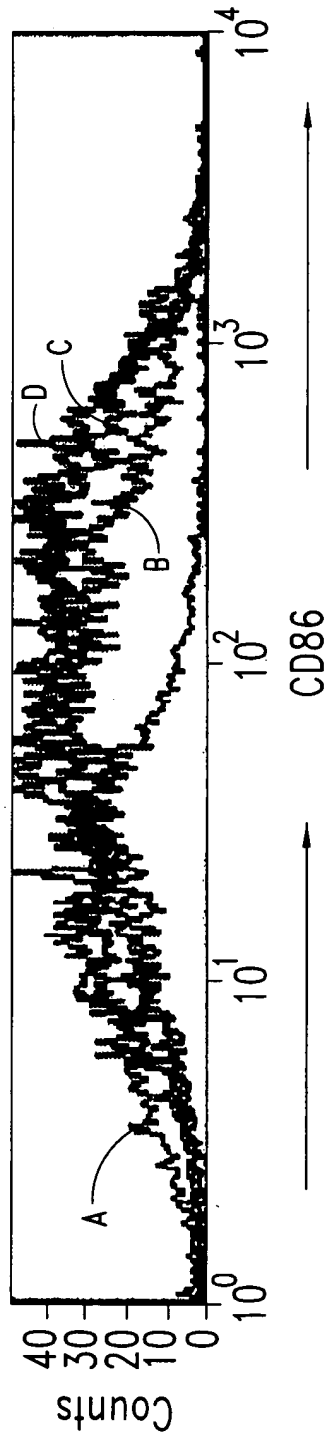


FIG. 4C

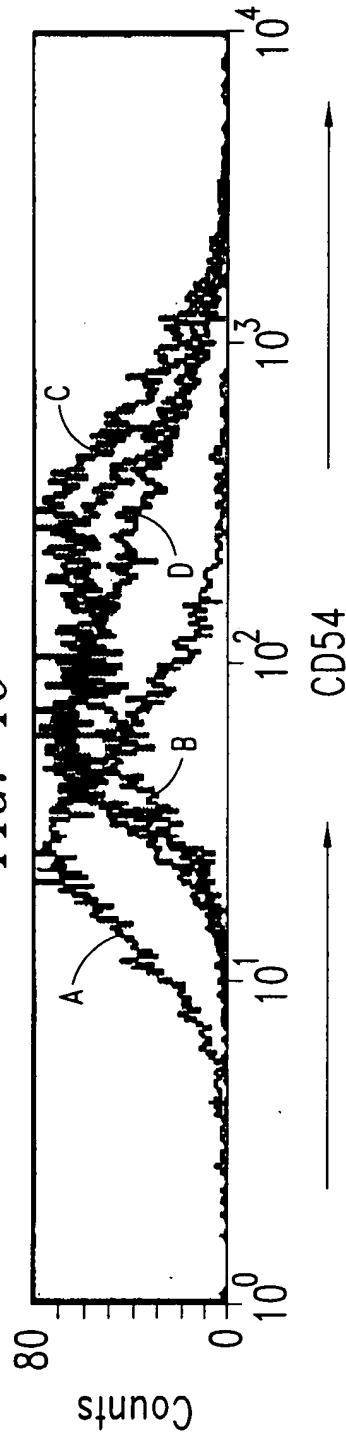


FIG. 4D

A= Leukemic B Cells Alone	B= Leukemic B Cells + Xcellerated T Cells T:B ratio=0.3:1	C= Leukemic B Cells + Xcellerated T Cells T:B ratio=1:1	D= Leukemic B Cells + Xcellerated T Cells T:B ratio=3:1
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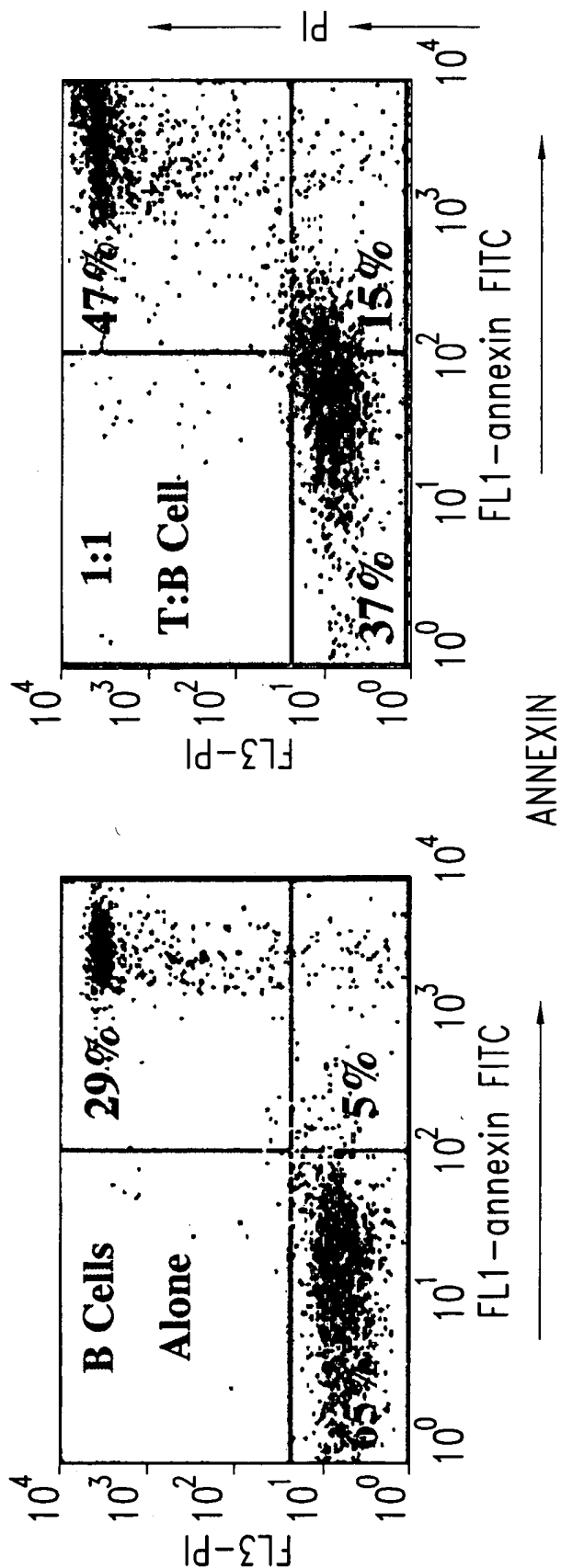


FIG. 5A

FIG. 5B

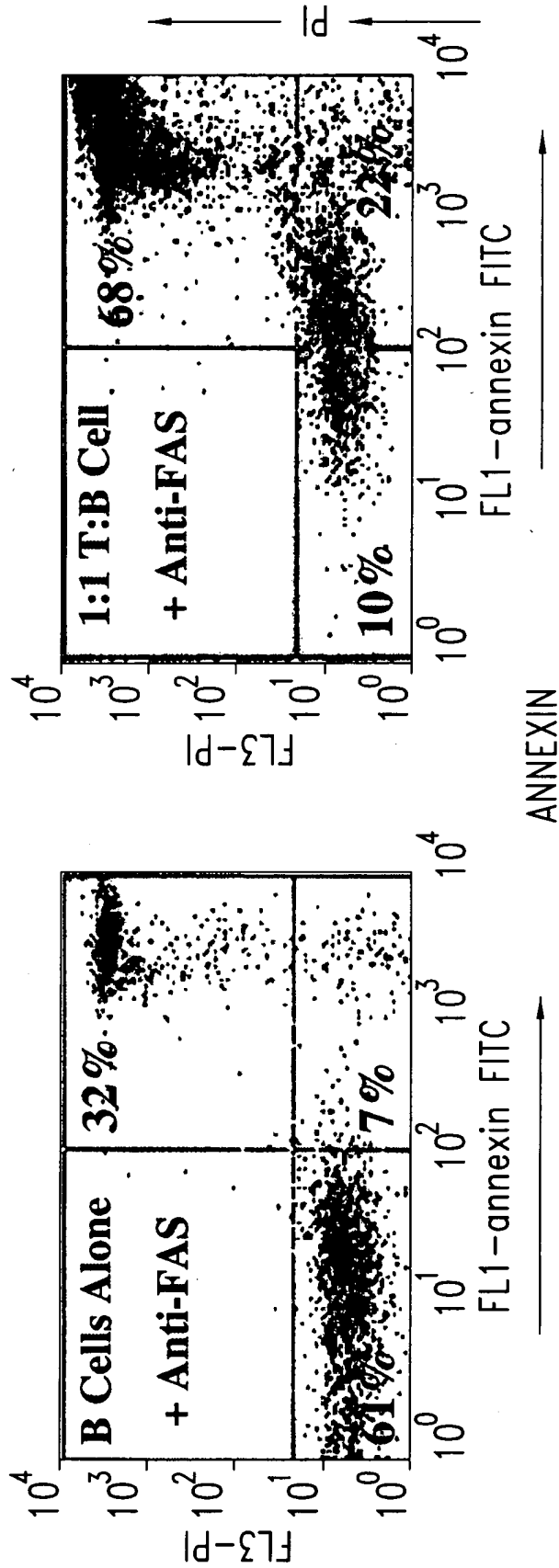


FIG. 5C

FIG. 5D



T Cells Grow and Tumor Cells Are Eliminated During the Xcellerate Process

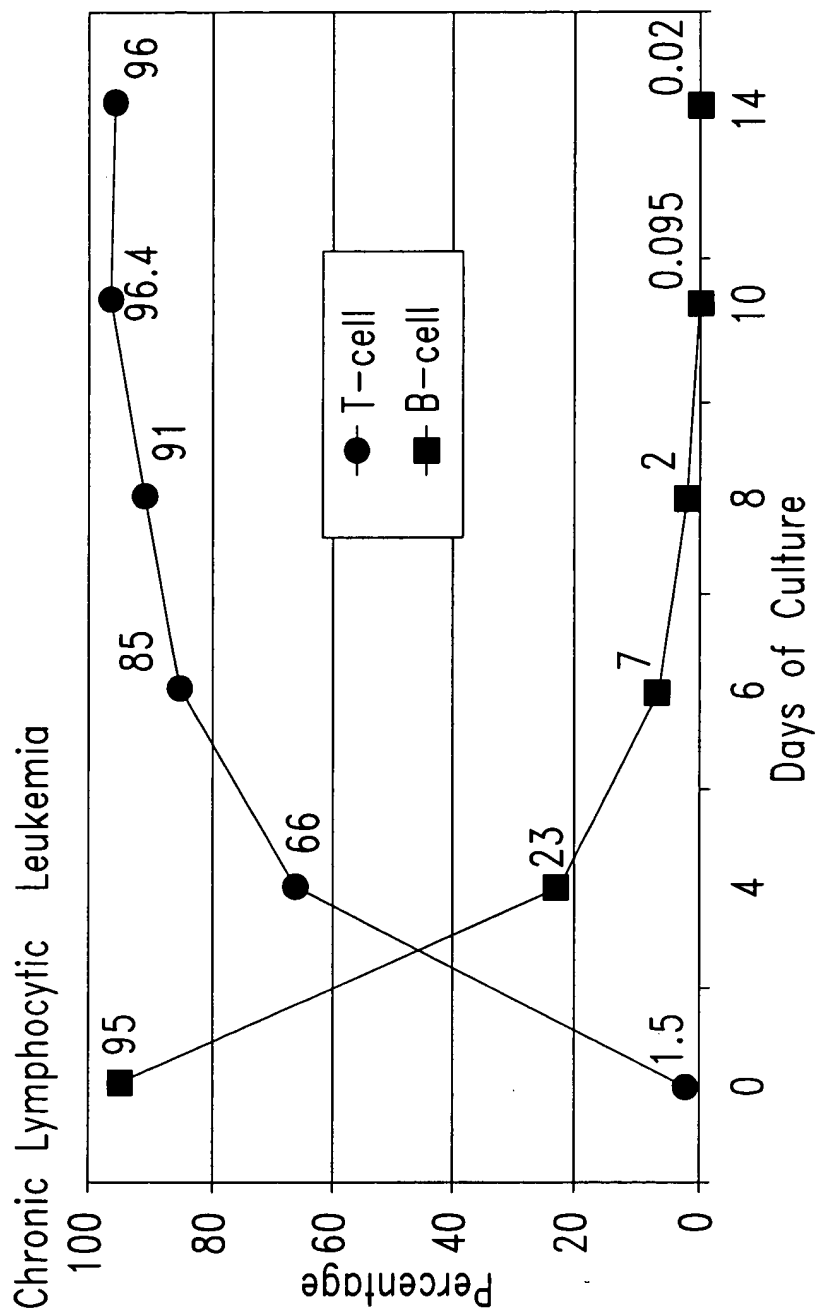


FIG. 6

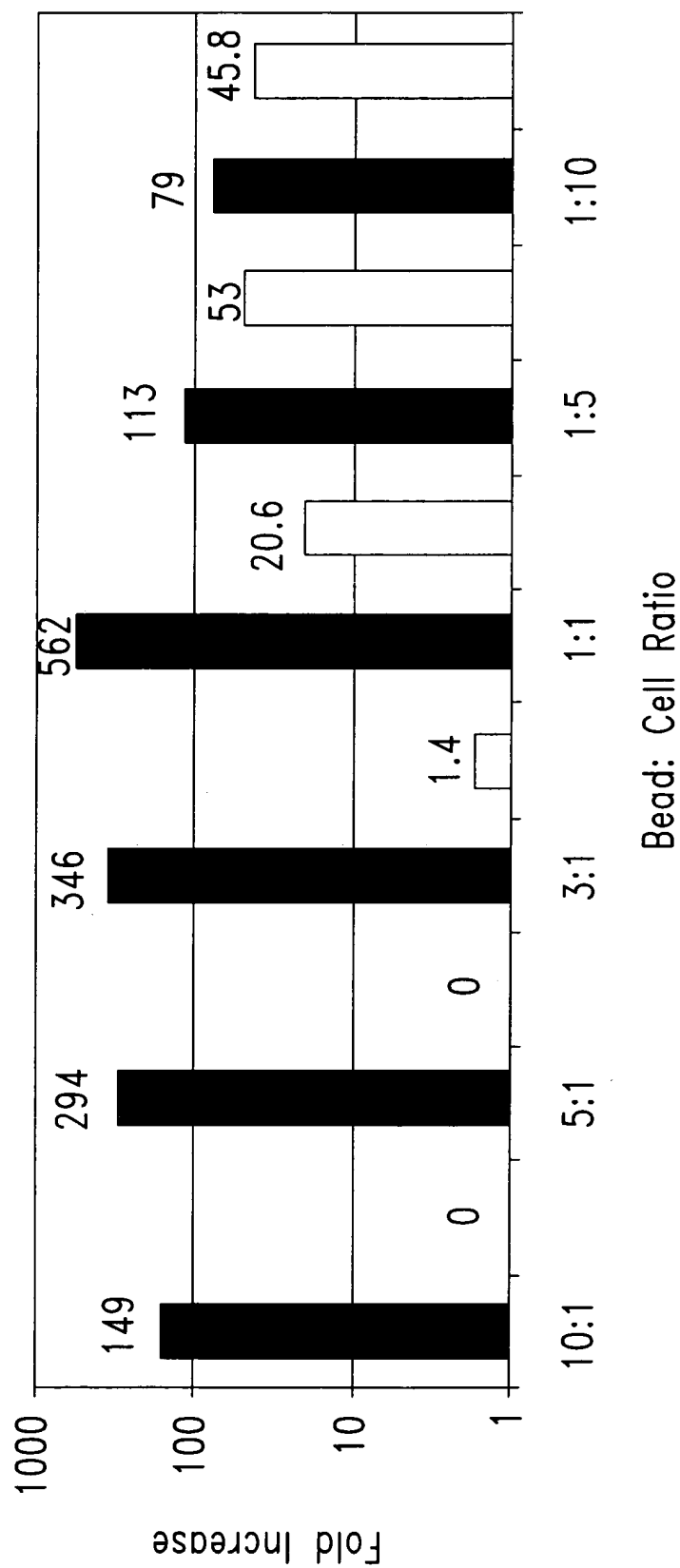
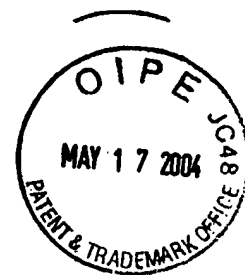


FIG. 7